Salicylazosulfapyridine (Salazopyrin or Azopyrin) in Rheumatoid Arthritis and Experimental Polyarthritis

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SUMMARY

Thirty patients with rheumatoid arthritis were treated with Salazopyrin® for periods from two months to one year.

Fourteen patients were symptomatically relieved in varying degrees. This group included seven patients not previously benefited by gold therapy and four who had had toxic reaction to gold. The sedimentation rates tended to remain elevated in spite of symptomatic improvement. Extension of disease to joints not formerly involved appeared in only one patient under treatment.

Continuation of small dosage for long intervals seemed advantageous in the small number of patients treated in this study.

Fourteen patients were not relieved symptomatically by Salazopyrin, but they did not become worse. This group included eight patients with severe, advanced disease, six of whom had not been benefited by chrysotherapy.

In one patient there was a moderate reduction in erythrocyte count and in hemoglobin. One patient refused medication, claiming extreme nervousness.

Salazopyrin is variably and comparatively poorly absorbed in man.

In experimental polyarthritis of rats, administration of 0.5 per cent Salazopyrin in the diet produced a slight beneficial effect, while 1 per cent made the infection worse. Changes in body weight and in leukocyte content in the blood of rats and mice showed Salazopyrin to have minimal toxic effect in these rodents.

THE sulfonamides have been extensively tried in the treatment of rheumatoid arthritis. In general, their use has been found ineffective in altering the course of the disease. There are, however, two notable exceptions in which the use of sulfonamides has been favorably reported. Parr and Shipton[6, 7] working in Australia reported the favorable effect of benzylsulfanilamide (proseptasine) in rheumatoid arthritis, in 1947, and again in 1949. Svartz, working in the Caroline Institute in Stockholm with the collaboration of the chemists Askelöf and Willstaedt, developed a di-azo dye, the formula of which is 4-(pyridyl-2-amidosulfonyl)-3'-carboxy-4'-hydroxy-azobenzene. This dye, known by the trade names Salazopyrin® and Azopyrin,® has been reported to be effective in the treatment of rheumatoid arthritis[10] and ulcerative colitis.[1, 12] The use of the sulfonamides and salicylates concurrently gave no favorable result, but it was postulated that a di-azo compound containing both of these substances might be deposited in heavy concentration in the connective tissue where the primary pathological involvement was apparent.

Salicylazosulfapyridine is a brownish yellow dye which is almost insoluble in water and other solvents. It is absorbed from the intestinal tract and partly broken down in the tissues and partly excreted in the urine as such. Its concentration in the urine may be determined colorimetrically, since in alkaline urine its presence gives rise to a bright orange-yellow color. In acid urine the dye causes no change in color. Svartz claims that the drug, when broken down, forms small amounts of aminosalicylic acid and sulfapyridine. Helander has shown that Salazopyrin, like other acid-azo compounds, has a special affinity for the connective tissues in mice,[5] as shown by fluorescent microscopy.

The purpose of the present study has been to determine the therapeutic value of Salazopyrin in rheumatoid arthritis of various degrees of severity and in the experimental polyarthritis (produced by L-4 strain of the pleuropneumonia-like organism) of rats, and to determine possible toxic effects in man, rats and mice.

THERAPEUTIC RESULTS IN RHEUMATOID ARTHRITIS

Thirty patients with rheumatoid arthritis of varying duration and severity were treated with Salazopyrin. Fourteen of these patients showed some measure of clinical improvement following the use of the

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TABLE 1.—*Summary of Cases in Which Improvement Was Shown on Salazopyrin Therapy*

Patient	Sex	Age	Duration of Symptoms (months)	Extent of Disease Prior to Treatment	Sed. Rate Before Treatment (mm. per hr.)	Daily Dosage and Duration of Salazopyrin Therapy	Symptoms of Toxicity	Status After Treatment	Sed. Rate After Treatment (mm. per hr.)
1.	F	29	12	Morning stiffness, swollen wrists, knees and ankles, unable to work.	34	4 gm. 3 wk.; then 3 gm. 12 wk.; then none for 2 wk.	Slight nausea, dizziness, thirst, restlessness.	Pronounced improvement in 1 wk.; after 4 mo. only slight morning stiffness of hands; now working.	30
2.*	M	29	60	Moderate ankylosing spondylitis with pain and swelling in hands, knees and shoulders; working.	21	4 gm. 2 mo.; then 2 gm. 10 mo.	Nausea relieved by sodium bicarbonate.	Less fatigue, less pain and stiffness, no extension of disease; working.	15
3.*	M	32	18	Moderate ankylosing spondylitis with great pain and swelling, knees and wrists, unable to work.	36	2 gm. 6 wk.; none 3 wk.; 1.5 gm. 3 mo.	Decrease in R.B.C. and Hgb. after 6 wks.	Gain in wt.; less stiffness and swelling; generally better; working; 2 new joints involved.	44
4.	F	64	36	Mixed arthritis, swollen, painful knees, able to do housework.	30	3 gm. 2 wk.; then 1.5-2.0 gm. 4 mo.	Drowsy, sleepy and nauseated on the higher dosages.	Asymptomatic; working.	23
5.*	M	27	252	Ankylosing spondylitis with swollen knees and wrists; able to walk and do little work.	30	4 gm. 6 wk.; 3 gm. 2½ mo.	None.	Pronounced improvement; weight gain; less tired; no swelling of knees; working full time.	26
6.*	F	38	120	Pronounced morning stiffness, pain in hands, shoulders and left hip; working.	46	2-2.5 gm. 6 mo.; then 3 gm. 2 wk.; then 1.5 gm. daily 4 mo.	Nausea relieved by sodium bicarbonate.	Moderate improvement; less stiff and tired, less pain; no progression of disease; working.	30
7.*	M	53	168	Ankylosing spondylitis with involvement of wrists, shoulders, hands, knees, feet; not working.	48	2.0 gm. 6 wk.; 3 gm. 1 mo.; 2 gm. 4 mo.	Nausea after large doses.	Gained 10 lbs.; less tired and stiff; greater range of motion; working.	30
8.†	F	57	48	Mixed arthritis, great pain, wrists, knees, feet and hips; unable to work.	40	2 gm. 2 mo.	None.	No pain; slight morning stiffness of hands; doing housework.	32
9.	F	31	72	Early involvement sacro-iliac, pain in hips and shoulders; working.	17	1.5 gm. 2 days; 0.25 gm. 1 wk.; 3 gm. 2 mo.	Occasional nausea.	More energy; less stiffness; less pain in back and joints; working.	12
10.*	F	29	24	Severe generalized rheumatoid arthritis with marked pain and stiffness; able to walk.	24	0.25 gm. 1 wk.; 3 gm. 1 mo.; 2 gm. 7 mo.	None.	Gained 15 lbs.; still has considerable pain, but no swelling; good range of motion; little stiffness; not enough rest; does housework.	34
11.†	F	51	54	Mild rheumatoid arthritis with hands, one shoulder and ankle involved; working.	24	4 gm. 1 mo.; 3 gm. 3 mo.; 2 gm. 9 mo.; none for 3 mo.	Slight dizziness after higher doses.	Rapid striking relief of symptoms; no swelling or pain; little stiffness; working.	18
12.†	M	40	180	Pronounced morning stiffness, pain and swelling knees, hands and shoulders; working.	40	2 gm. 1½ mo.; 3 gm. 4 mo.; 1 gm. 7 mo.	Occasional nausea.	Symptom-free; working.	?
13.*	M	24	18	Pronounced joint deformity, pain, swelling, all joints involved; in bed.	38	4 gm. 2 mo.; 3 gm. 9 mo.	Slight nausea after larger doses.	Gain in wt. and strength; no progression; less stiffness; greatly improved; walks daily.	21
14.†	F	24	60	Ankylosing spondylitis and swelling of knees, ankles and wrists; working.	12	2 gm. 2 mo.	None.	Feels warmer; less fatigue; less swelling and pain; working.	?

*No benefit from previous gold therapy.

†Benefited symptomatically by previous gold therapy but toxic reaction to gold developed.

drug. In 16 other patients the drug was discontinued either because of lack of clinical response or because of varying degrees of toxic reaction.

Table 1 shows brief summaries of the cases in which the patients were improved while taking Salazopyrin. The duration of therapy varied from two to 12 months. With one exception all patients are still on small maintenance doses of the drug, usually 1.0 to 2.0 gm. daily. Due to difficulties in the supply of Salazopyrin, there were two intervals of three weeks each during which most of these patients received no therapy other than salicylates. During these intervals all patients became worse symptomatically and showed much more stiffness, pain and joint swelling. The nausea which developed on higher doses in these cases was minimal and was relieved by sodium bicarbonate. These patients all noted pronounced improvement within the first seven to 14 days of therapy. They had all been taking large quantities of salicylates prior to the Salazopyrin, and after several days most of them did not have to take salicylates. Eleven of these patients had received previous chrysotherapy. Four of them had shown improvement with gold but toxic reactions necessitated its discontinuance. Seven of them showed no benefit from previous gold therapy. In none of these cases was there any obvious "focus of infection" from which streptococci or other bacteria were cultured.

In another group of 14 patients the use of Salazopyrin was without any demonstrable benefit. This group included eight patients with severe advanced rheumatoid arthritis (one of these was later relieved of symptoms with ACTH and cortisone). Six of these eight patients had shown no benefit with prolonged chrysotherapy and two of them had to give it up because of toxic reactions. In this group of 14 patients there were also three with mild mixed arthritis of the knees and hips, one with early ankylosing spondylitis, and two with early typical rheumatoid arthritis. Among the patients in this group there was little evidence of toxic reaction to Salazopyrin except for nausea when the daily dosage exceeded 3 gm. In one case a skin rash developed but it subsided on withdrawal of medication. This was not accompanied by a reduction in erythrocytes in the blood.

Two other patients were given Salazopyrin. One woman, aged 57, who had rheumatoid arthritis of 20 years' duration refused Salazopyrin after two days because of "nervousness" which developed subsequent to its use. In the other case, that of a woman, aged 27, who was taking 3 gm. of Salazopyrin daily, there was a reduction in erythrocytes in the blood from 4,920,000, with 15.5 gm. hemoglobin, to 3,860,000 with 12.4 gm. hemoglobin, in eight days. Withdrawal of Salazopyrin and the use of liver extract parenterally was followed by an increase in erythrocytes to former levels in two weeks. This was the only evidence of toxic effect on the hematopoietic system in any of the 30 cases in this study.

CLINICAL TOXICITY

With the exception of the single case of decreased erythrocyte count and hemoglobin, there was no change in the blood picture in these 30 cases. There was generally a decrease in the per cent of banded young forms of polymorphonuclear neutrophils coincident with clinical improvement.

Several patients complained of restlessness and sleeplessness while taking 3 gm. or more of Salazopyrin daily. Mild nausea was the principal toxic manifestation; it occurred in all patients. In one case skin rash developed; it promptly disappeared when medication was discontinued.

BLOOD LEVELS OF SULFAPYRIDINE AND SALICYLATE

Blood levels of sulfapyridine and salicylate were determined in several patients (Table 2). Relating these blood levels to dosage revealed uneven absorption varying with the individual. In one patient given 2.5 gm. of Salazopyrin daily there was no demonstrable sulfapyridine or salicylate in the blood, while in another patient a sulfapyridine blood level of 4.0 mg. was obtained on a daily dosage of 4.0 gm.

TABLE 2.—*Blood Levels of Sulfapyridine and Salicylate in Patients Taking Salazopyrin*

Sex	Daily Dose (gm.)	Interval Between Medication and Test (hours)	Sulfapyridine Blood Level (mg./100 cc.)	Salicylate Blood Level (mg./100 cc.)
F	3.0	4	1.3	7.0
M	2.5	3	0	0
F	3.0	3	0.8 mg.	0
F	3.0	3	2.2	not determined
F	4.0	2	4.0	not determined
F	3.0	3	1.1	not determined
F	1.5	2	0.55	not determined

EFFECTS OF SALAZOPYRIN IN POLYARTHRITIS OF RATS

Salazopyrin was ground into the food (Purina Laboratory Chow®) of albino rats averaging 60 to 80 gm. in body weight. The rats were given intraperitoneal injections of the L-4 strain of pleuropneumonia-like organism which produces a polyarticular arthritis in five to seven days. Arthritis of this type in rats has been used as a method of testing various chemotherapeutic agents in relation to gold salts which will prevent or cure this arthritis.[2, 8, 13]

Scoring the effect of the medication includes three factors—survival rate, incidence of arthritis, and the maximum average arthrogram score. The arthrogram is a scoring device permitting the assignment of numerical values corresponding to the extent of the joint involvement.[14] The maximum average is computed by dividing the number of rats in the group into the total of the individual arthrograms on the day of most extensive joint involvement.

Table 3 shows the effect of Salazopyrin on the polyarthritis of rats. A dosage of 0.5 per cent Sal-

TABLE 3.—*Effect of Salazopyrin on Polyarthritis of Rats*

Trial	Concentration in Diet (per cent)	No. of Rats	Max. Avg. Arthrogram Score	Incidence (per cent)	Survival (per cent)
A	0.5	20	2.07	66	95
A	0 (control)	20	2.15	77	64
B	1.0	20	1.95	75	100
B	0 (control)	20	1.15	50	95
C	1.0	20	1.5	50	100
C	0 (control)	20	0.87	35	100
D	3.0	20	0.4	26.5	95
D	0 (control)	20	0.2	10	100

azopyrin in the diet showed a mild beneficial effect in decreasing the incidence of arthritis, increasing the survival rate and slightly decreasing the maximum average arthrogram score. A dose of 1 per cent in two separate experiments showed that in the treated animals the incidence of arthritis was higher and the disease more extensive. Separate control values are presented for each group, since the pathogenicity of the microbes varies from one sub-culture to another.

TESTS FOR TOXIC EFFECTS OF SALAZOPYRIN

Body Weight Changes in Rats and Mice. Five albino male rats (average weight 92.2 gm.) and five albino female rats (average weight 96.6 gm.) were placed on 1 per cent Salazopyrin ground into the diet. Five males (average weight 94.8 gm.) and five females (average weight 94.8 gm.) were placed on the regular diet (Purina Laboratory Chow). After an interval of 69 days the males taking Salazopyrin averaged 215 gm. in weight and the females 152 gm. The male controls averaged 236 gm. and the female controls 167 gm.

Five male albino mice (average weight 18.6 gm.) and five females (average weight 17.4 gm.) were placed on 1 per cent Salazopyrin ground into the diet. Five male controls averaged 17.4 gm. body weight and the five female controls averaged 16.2 gm. After 18 days the males on Salazopyrin averaged 30.5 gm. in body weight and the females 24.0 gm. Those on the regular ground diet (Purina Laboratory Chow) averaged 26.2 gm. for the males and 32.4 gm. for the females.

Blood Sulfapyridine Level in Rats. A determination of the blood sulfapyridine level on the pooled blood of four rats on the 1 per cent Salazopyrin diet showed a concentration of 1.029 mg. per 100 cc.

Blood Counts. Rats on 1 per cent Salazopyrin diet showed the following average leukocyte counts:

	Males	Females
13th day on Salazopyrin	16,800	11,920
(Controls)	12,000	11,920
68th day on Salazopyrin	18,400	13,080
(Controls)	15,500	15,100

Mice on 1 per cent Salazopyrin diet showed the following average leukocyte counts:

	Males	Females
121st day on Salazopyrin	13,025	9,787
(Controls)	6,760	4,285

Differential blood cell counts averaged as follows for the rats on 1 per cent Salazopyrin diet:

13th day on the diet (per cent):

	Salazopyrin Males	Salazopyrin Females	Control Males	Control Females
Neutrophils	9	9	10	12
Eosinophils	0	1	0.5	0.5
Basophils	0	1	0.5	0.5
Lymphocytes	91	85	82.0	86
Monocytes	0	4	7.0	1

68th day on the diet (per cent):

	Salazopyrin Males	Salazopyrin Females	Control Males	Control Females
Neutrophils	9	6.6	17.0	23.0
Eosinophils	1	0	5.5	1.0
Basophils	0	0.4	1.5	1.0
Lymphocytes	89	92.6	73.0	75.0
Monocytes	1	0.4	3.0	0

The average differential blood cell counts on the mice medicated with 1 per cent Salazopyrin for 121 days are given below:

	Salazopyrin Males	Salazopyrin Females	Control Males	Control Females
Neutrophils	12.5	21	18	11.8
Eosinophils	0	0	0	0
Basophils	0	0	0	0
Lymphocytes	85.5	77.5	80	86.4
Monocytes	2.0	1.5	2	1.8

In the rats after 68 days on Salazopyrin the neutrophil counts averaged about one-half to one-third those of the unmedicated controls. The percentage of lymphocytes was slightly higher among those rats treated than among the controls. In the mice after 121 days on Salazopyrin the treated males showed fewer neutrophils than the controls and the females showed more than the controls.

COMMENT

While the cause of rheumatoid arthritis has not been determined, a number of agents have been employed empirically with variable success in moderating its severity. Cortisone acetate and ACTH have been used recently with considerable success in altering the so-called rheumatic state in man, but the mechanism of the action of these agents is not yet fully understood. The use of chrysotherapy in typical rheumatoid arthritis is also often followed by remissions in the severity of the disease. Recently two salts of copper have been advocated by Forestier and his associates in the treatment of this disease.[3] In the published reports on benzylsulfanilamide and Salazopyrin[6, 7, 10, 11, 12] some therapeutic merit is also claimed. A recent study of Salazopyrin made in England by Sinclair and Duthrie[8] in 20 cases of rheumatoid arthritis resulted in the conclusion that the drug was of no specific use in the disease. The difference between that conclusion and the results of the study herein reported may have been due to the fact that in the present study the dosage was smaller and was continued for longer

periods. Sinclair and Duthrie also noted little toxic reaction to the drug. A further observation was that the accelerated sedimentation rate did not tend to return to normal under treatment.

The picture is confusing when agents of such varying composition seem capable of altering the course of rheumatoid arthritis. In this confusion it is not wise to shift all the emphasis of current research to evaluation of the hormones which are so spectacular in their effect on the disease. The implication that certain heavy metals and sulfonamides may, in some way, produce an effect similar to in kind but of different degree than that produced by these hormones indicates the desirability of studying the mode of action of these agents. Salazopyrin does not produce as many remissions as does chrysotherapy, but it is much less toxic, and, when its beneficial effect is manifest, the result seems more prompt.

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